FORESTRY

FRDA REPORT 142

DIEBACK OF CONTAINER-GROWN
DOUGLAS-FIR SEEDLINGS

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FOREST RESOURCE DEVELOPMENT AGREEMENT

Canada

BC

FRDA REPORT 142

DIEBACK OF CONTAINER-GROWN DOUGLAS-FIR SEEDLINGS

by
L.D. Husted
S. Barnes

Contractor: Canadian Pacific Forest Products Ltd.
8067 E. Saanich Road, RR 1
Saanichton, B.C.

Departmental Representative:

Dr. J. Sutherland
Pacific Forestry Centre
Forestry Canada
506 W. Burnside Road
Victoria, B.C.

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ABSTRACT

Dieback of container-grown Douglas-fir seedlings is caused by a minor root pathogen, Pythium. Possible sources of Pythium in nursery mixes include peat, water, air-borne spores and reuse of contaminated styroblocks. The incidence and severity of dieback damage are strongly influenced (1) by seedling susceptibility to the pathogen, which is highest the first few weeks after germination, and (2) by the environmental conditions, particularly the moisture content, temperature and pH of the growing mix.

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A. INTRODUCTION

This report summarizes the second year of a two year study on Douglas-fir dieback. For the past five years, nursery growers have observed a growth problem, termed needle dieback, in container Douglas-fir seedlings. Dieback symptoms, including stunted shoot growth, needle chlorosis, and dieback of needles from the tips to the bases, are first noticed when the seedlings are a few centimetres tall. Dieback seedlings rarely meet the minimum cull standards set for planting stock.

Dieback occurs in patches throughout Douglas-fir seedlots and may result in cull losses of 0% to 25% depending upon the nursery, sowing date and year. High air and growing medium temperatures during the germination period appear to increase the incidence of dieback. Weather conditions, therefore, may be responsible for some of the observed variation in dieback incidence.

In 1986 at the time this research was initiated, the consensus of opinion was that dieback might be caused by one or more of the following factors: (1) toxic levels of ammonium in the growing medium, (2) a calcium or boron deficiency, (3) high medium temperatures, or (4) poor medium aeration. Disease was not considered a cause of dieback because dieback seedlings had roots that appeared healthy and free of the rotting symptoms normally associated with disease.

In the first year of this research (1986-87), summarized in a previous file report (Husted and Barnes 1987), we studied the relationship of Douglas-fir dieback to growing medium properties and date of sowing. This research showed that peat source, perlite, vermiculite, and slow-release fertilizer had no significant impact on the incidence of dieback. In fact, slow-release fertilizer at three times the normal rate did not induce dieback symptoms. Peat dilution with perlite (to create a very porous medium) also had no effect on dieback incidence. Although the incidence of dieback appeared to increase with hot, sunny weather, temperature alone could not explain the high degree of variability in dieback incidence which occurred between adjacent styroblocks.

The 1986-87 research also showed that the major initial impact of dieback was on root growth and not needle growth; the root weight and root hair development of dieback seedlings were severely reduced. Nutrient concentrations and contents of dieback seedlings were not indicative of ammonium or aluminum toxicities, anaerobic growing medium conditions, or insufficient transport of calcium or boron to needle tips. Nutrient concentrations were higher in dieback seedlings compared to healthy seedlings, mainly because of the poorer dry weight growth of dieback seedlings.

Results of the 1986-87 research suggested that dieback was caused by Pythium, a minor root pathogen. Pythium was consistently isolated from the roots of dieback seedlings. Galaaen and Venn (1979) isolated Pythium from Norway spruce seedlings with symptoms similar to those of Douglas-fir dieback, i.e., stunting and dieback of needle tips. The specific identity and pathogenicity of the organism isolated from dieback seedlings are unknown. However, there are numerous reports of subclinical damage caused by a variety of Pythium species (Hodges 1985, Hershman et al. 1986, Kobriger and Hagedorn 1984). Subclinical damage is characterized by stunting and growth losses in plants which have normal-appearing roots and shoots.

Yield reductions due to minor root pathogens are receiving more attention from horticulturists (Salt 1979). Some of these yield reductions have been associated with root-invading fungi such as Pythium which do not cause external root rot lesions. Stanghellini and Kronland (1986) reported that Pythium dissotocum was responsible for yield reductions of 12% to 54% in lettuce crops in the absence of visible root rot symptoms. The degree of damage caused by minor root pathogens typically depends on the growing environment and host plant vigour (Salt 1979). The severity of damage to lettuce in Stanghellini and Kronland's (1986) study, for example, correlated with the growing regime temperature.

B. OBJECTIVES

We concluded from the 1986-1987 research that the presence of Pythium coinciding with unfavourable cultural regimes reduces seedling vigour and causes dieback. The objectives for research in 1987/88 were:

(1) to determine the pathogenicity and identity of the Pythium isolated from dieback seedlings;

(2) to investigate alternative explanations (to the pathogen hypothesis) for the sterilization effect, i.e., steam sterilization reduces the incidence of dieback, and

(3) to examine the effects of cultural practices and the growing environment on the incidence of dieback.

C. PATHOGENICITY OF *PYTHIUM* SPP. ISOLATED FROM DIEBACK SEEDLINGS

INTRODUCTION

Koch's postulates were followed to determine the pathogenicity of the organisms isolated from dieback seedling roots. Testing for pathogenicity using the Koch's postulates involves the following.

(1) Confirming that diseased plants are always associated with the suspect pathogen.

(2) Isolating, culturing and maintaining the suspect organism in pure culture.

(3) Inoculating healthy seedlings with the suspect pathogen. The inoculated seedlings should develop the typical dieback disease symptoms.

(4) Isolating the suspect pathogen from the inoculated plants which exhibit disease symptoms.

(5) Confirming the identification of this isolated organism by microscopic examination and/or re-inoculation of healthy plants.

Methods used to test Koch's postulates are described in Appendix A.

RESULTS

The percentage of seedlings with visible shoot dieback symptoms ranged from five to ten percent in two replicates of Koch's postulates. Seedlings with dieback symptoms (greenish stems, curled straw-coloured needles, and stunting) appeared in each of the *Pythium* inoculum treatments with the exception of the inoculum prepared from isolate #7116. No dieback symptoms occurred in the controls. In general, control seedlings had more well-developed fibrous root systems than *Pythium*-inoculated seedlings.

Pythium was isolated from cleared roots of seedlings showing dieback symptoms. *Pythium* was also isolated from healthy-appearing seedlings inoculated with *Pythium*. No *Pythium* was isolated from cleared roots of control seedlings. Microscopic examination of the *Pythium* oospores isolated from inoculated dieback seedlings showed they were similar to the oospores of the original inoculum cultures. Dr. H. Hartmann, M.B. Research and Development Ltd., has identified the dieback pathogen as *Pythium ultimum*.

We repeated the Koch's postulates testing using a longer incubation period (4-6 weeks) hoping to increase the percentage of inoculated seedlings showing dieback symptoms. The most active portion of the laboratory inoculum is the hyphae. However, these hyphae are aseptate and easily damaged by mixing with PVM. An incubation period may be necessary to build-up an active population of pathogenic Pythium from the oospore component of the inoculum. Incubation of Pythium oospores for up to six weeks promotes their germination (Ayers and Lumsden (1975). Drechsler (1952) and Zentmyer and Erwin (1970) observed that a period of after ripening is required for oospore germination of Pythium ultimum.

Unfortunately, the longer incubation period did not increase the incidence of dieback. Therefore, we decided to focus the remainder of our research time and money on the other 1987/88 objectives rather than repeating the Koch's postulates with further modifications to increase the incidence of dieback. Koch's postulate testing for minor root pathogens is difficult because damage to host plants by minor root pathogens depends greatly upon environmental conditions and the age and vigour of the host (Salt 1979). For example, successful germination and infection of seedling root systems are dependent on temperature (Ayers and Lumsden 1975) and root exudates (Kraft 1974). Our later research (section H) showed that growing mix temperature, pH and moisture regime have a significant impact on the development of dieback symptoms.

D. ALTERNATIVE EXPLANATIONS FOR THE STERILIZATION EFFECT

The 1986/87 dieback research showed that medium sterilization reduced the incidence of dieback. We felt that sterilization reduced Pythium inoculum in the growing medium and therefore dieback incidence. However, there were other possible explanations for the sterilization effect: (1) an increase in the populations of microorganisms antagonistic to Pythium, (2) inhibition of microbial toxins, (3) a decrease in microbial competition, (4) changes in chemical composition of the medium, particularly available nitrogen, or (5) a combination of these factors.

The original research plan for 1987/88 was to complete objective one, i.e., to determine the pathogenicity of the Pythium isolated from roots of dieback seedlings before proceeding with further research. We hypothesized that the sterilization effect was mainly due to inhibition of a pathogen. The results of

experiments conducted in 1986/87 suggested that water-soluble toxins or changes in the physical or chemical properties of the media were not major causes of the sterilization effect. A decrease in microbial competition for oxygen or nutrients was considered an unlikely explanation for the sterilization effect because the growth of seedlings in sterilized PVM was not significantly greater than that of healthy seedlings in nonsterile PVM.

However, we decided to re-examine alternative explanations for the sterilization effect on dieback before the completion of the pathogenicity experiments because the Koch's postulate testing was taking longer than planned.

Several experiments were conducted to re-examine the alternative explanations. These experiments are reported in Appendix B. Briefly, the results are as follows.

(1) Water-soluble toxins do not cause dieback.

(2) Sterilization of the growing mix does not significantly alter the availability of nitrogen.

(3) Sterilization does significantly increase the populations of organisms such as Penicillium and Trichoderma known to be antagonistic to minor root pathogens. Therefore, sterilization of growing mixes may increase the level of antagonism to disease organisms such as Pythium in addition to reducing or eliminating Pythium propagules.

E. INFLUENCE OF NURSERY GERMINATION REGIME ON DIEBACK INCIDENCE

INTRODUCTION

Some concern was expressed during the 1986/87 dieback research that the CPFP nursery germination regime (e.g., temperature or watering regimes) differed from other nurseries in a way which promoted dieback. To address this concern, we decided to send styroblocks filled with dieback medium and sown with Douglas-fir SL# 7276 to three other coastal container nurseries selected in consultation with G. Matthews (MOF) to represent a wide range of germination and growing regimes for Douglas-fir. To avoid possible contamination of the nurseries, the styroblocks and medium were returned to the CPFP nursery at the conclusion of the experiment.

METHODS

Peat:vermiculite medium was collected from blocks containing dieback seedlings in 1986, and production peat:vermiculite medium was added to increase the volume of dieback medium. Then the medium was incubated in a plastic pail at room temperature (approximately 21°C) for several weeks. This dieback medium was loaded by hand into new 313 styroblocks to a bulk density of 0.09 g/cc. Microbial analysis of the dieback medium at this time showed 1800 CFU/gram (fresh weight) of Pythium spp.

Douglas-fir germinants were transplanted (one per cavity) into the styroblocks and two styroblocks were distributed to four different nurseries: Green Timbers (B.C. Ministry of Forests nursery, Surrey), Koksilah (B.C. Ministry of Forests, Duncan), MacMillan Bloedel Ltd. (Nanaimo) and CPFP Ltd. (Saanichton). Each nursery placed the blocks in operational greenhouses with newly germinating seedlings. Eight weeks after transplanting, the numbers of healthy and dieback seedlings were recorded for each styroblock.

RESULTS

The average incidence of dieback was similar for three of the nurseries, including the CPFP nursery (Table 3), showing that a germination regime specific to the CPFP nursery was not responsible for the higher incidence of dieback observed in some years at this nursery.

Table 1. The average percentage of surviving seedlings with dieback symptoms at each nursery location (average of two styroblock

Nursery	% Dieback
Green Timbers	82
MacMillan Bloedel	45
Koksilah	59
CPFP Ltd.	49

F. REUSE OF STYROBLOCKS AND DIEBACK INCIDENCE

INTRODUCTION

Possible sources of *Pythium* inoculum in a nursery include peat, pond water (Dennis 1988) or wind-blown soil. Once in a nursery, we hypothesized it may be also be transmitted from year to year through the reuse of contaminated styroblocks. Dennis (1988) found that none of the commonly used styroblock washing procedures completely eliminated root pathogens.

METHODS

Styroblock Treatments

Six styroblocks containing dieback seedlings from a previous trial were cut in half. Three washing treatments were applied randomly: (1) cold water, (2) Safer's Demoss at 1:20 Demoss to water (3) steam sterilization at 80°C for three minutes. Two new half styroblocks were used as a control. Seventy cavities per half block were filled with sterilized peat:vermiculite mix and sown with SL #7276 (2 seeds per cavity). Microbial analysis of the sterile medium showed no *Pythium* was present.

Growing Regime

The blocks were placed in a Forestry Canada greenhouse on a 16 hour photoperiod with 21°C day/15°C night temperatures in November 1987. The relative humidity fluctuated with an average value of 65%. Two weeks after sowing, the light levels were supplemented with high pressure sodium lights. Two weeks after germination the seedlings were "stressed" (28°C maximum daily temperature) for 48 hours. No fertilizer was applied for the duration of the trial.

Monitoring

The styroblocks were observed for dieback symptoms weekly. Six weeks after germination, the seedlings were harvested to determine mean seedling root and shoot oven dry weight (80°C for 24 hours). Cleared roots were examined microscopically for hyphae and oospores. Scanning electron micrographs (SEM) were taken of the root systems of six-week-old seedlings grown in new styroblocks and those grown in water-washed blocks. Samples of roots for the SEMs were obtained by extracting the whole seedling plug very carefully and preserving it whole in formalin acetic acid (FAA) before preparation for the SEMs. This procedure minimized disturbance of the root system.

RESULTS

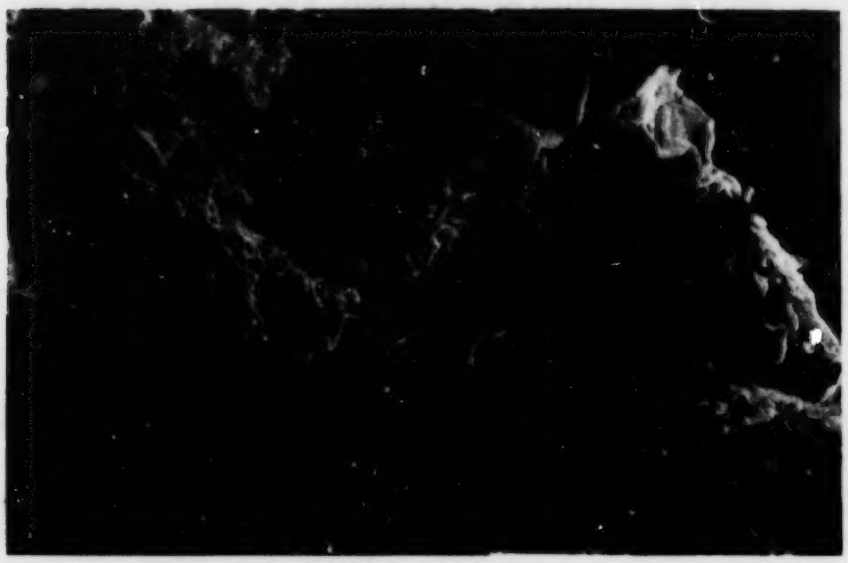
No dieback symptoms were observed in any of the treatment blocks. The treatments had no significant effect (at $p = 0.10$) on mean seedling shoot or root dry weight. There were qualitative differences observed, however, between root systems of seedlings grown in new styroblocks and those grown in water-washed styroblocks which previously contained dieback seedlings. Seedlings grown in new styroblocks were difficult to extract from styroblock cavities because of extensive root contact with the PVM. In contrast, the seedlings extracted from water-washed blocks were easy to pull from the cavities.

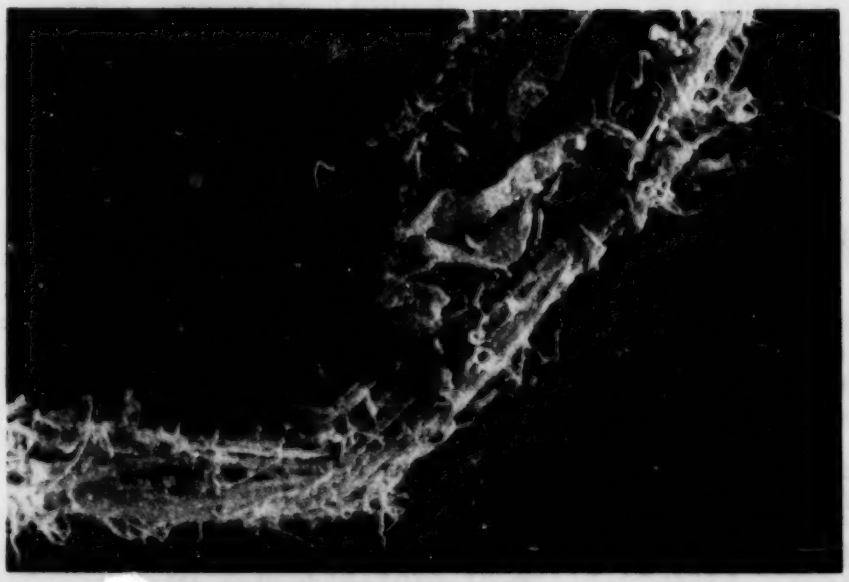
This qualitative difference was confirmed by the scanning electron micrographs (Figure 1). Root hair development (and root hair-growing mix contact) of seedlings grown in new styroblocks was much greater than that of seedlings grown in the water-washed styroblocks. These differences in root hair development were identical to the differences observed in SEMs of healthy and dieback seedling root hair development (Husted and Barnes 1987).

The weak root hair development of seedlings grown in water-washed styroblocks suggests that pathogens remaining in the old styroblocks may be reducing the root absorbing surface even if dieback symptoms are not produced. Minor root pathogens are generally restricted to juvenile tissues such as root hairs, root tips or cortical cells (Salt 1979). Damage to these tissues is easily overlooked and may only be of a temporary nature because most root hairs live only a few hours, days or weeks (Kramer and Kozlowski 1979). However, root hairs contribute to a significant proportion of the total root surface area and their importance for absorption of nutrients and water should not be ignored. Kozlowski and Scholtes (1948) found that the surface area of root hairs was 51% of the total root surface area in seven-week-old pine seedlings. Extensive damage to root hairs and other juvenile tissues may weaken a conifer germinant and reduce its resistance to transpiration stress or other pathogens.

Cleared roots of seedlings grown in new blocks had a few Pythium hyphae; roots of those grown in water-washed styroblocks contained both oospores and hyphae of Pythium. The presence of oospores indicates that Pythium activity had peaked and had entered a resting stage.

Figure 1. Micrographs of seedling roots grown in new styroblock (top photo) and from seedling grown in used, water-washed styroblock (bottom photo). The micrographs were prepared by Ms. Manning, Forestry Canada.





G. DIEBACK IN OTHER CONIFER SPECIES

MOF nursery personnel believe that dieback occurs in other species besides Douglas-fir (Mr. G. Matthews, MOF, pers. comm.). The objective of this experiment was to compare the incidence of dieback symptoms in Douglas-fir, lodgepole pine, white spruce, western redcedar and western hemlock seedlings grown in steam sterilized and unsterilized dieback medium.

METHODS

Growing Mix and Seedlings

Three styroblocks (313) of sterilized and unsterilized dieback medium were sown with stratified seed of five conifer species: lodgepole pine (SL# 2163), Douglas-fir (SL# 7276), western redcedar (SL# 3542), western hemlock (SL# 4087) and white spruce (SL# 29144). The five species were randomly assigned to cavities in each styroblock. Dieback medium was sterilized by autoclaving at 122°C for 40 minutes.

Growing Regime

Unfortunately, due to the date of project approval (June 1987) and the time required to obtain and stratify seed, this experiment was not started until September 1987. The seeds were germinated in a Forestry Canada greenhouse in mid-September 1987. Day temperatures fluctuated from 18°C to 24°C; night temperatures from 6°C to 10°C. Relative humidity ranged from 60% (day) to 80% (night). There was no supplemental lighting.

RESULTS

Only a few Douglas-fir seedlings showed dieback symptoms. The lack of symptoms in the other four species suggests these species are resistant to the pathogen in the dieback medium. However, the lack of symptoms may also reflect the poor growing conditions of the trial i.e., low light intensity, short photoperiod and cool temperatures. The white spruce germinants, for example, had formed buds six weeks after sowing.

This experiment was repeated in the spring of 1988 at the CPFP container nursery. The growing regime was favourable (i.e.,long photoperiod and warm, sunny weather) for the occurrence of dieback.

The results were similar to those of the first experiment; dieback symptoms (needle dieback and stunting) occurred in 13% of the Douglas-fir germinants but not in any of other species. Also Pythium was isolated from the root systems of the Douglas-fir germinants but not from the roots of other species.

H. EFFECTS OF ENVIRONMENTAL CONDITIONS ON THE INCIDENCE OF DIEBACK

GROWING MIX TEMPERATURE AND MOISTURE REGIME

Introduction

An important characteristic of minor root pathogens is that damage to the host plant is influenced strongly by environmental conditions and the vigour of the host plant (Salt 1979). Soil moisture and temperature are considered the most important environmental factors influencing the severity of diseases caused by Pythium spp. (Hendrix and Campbell 1968). Pythium spp. which produce zoospores appear to be influenced more by soil moisture than by soil temperature; other species tend to be influenced more by soil temperature (Biesbock and Hendrix 1970). Pythium ultimum, the species thought to cause dieback, does not readily produce zoospores; it grows under a wide range of soil moisture and temperature conditions (Hendrix and Campbell 1968, Krupa and Dommergues 1979).

The effects of temperature and moisture on Pythium disease may be related more to the influence of these factors on the growth and physiology of the host plant (Leach 1947,Thomson et al. 1971) or on the activity of soil microbial populations (Chen et al. 1988, Cook and Baker 1983, Hershman et al. 1986) than to their direct action on Pythium inoculum potential. Leach (1947) studied the preemergence infection of a variety of horticultural crops by Pythium spp. over a range of soil temperatures. In all combinations of host and pathogen examined by him, preemergence infection was severest at soil temperatures relatively less favourable to the host than to Pythium.

Chen et al. (1988) demonstrated a high correlation between microbial activity and suppression of damping-off disease caused by P. ultimum. Pythium spp. tend to be pioneer colonizers of substrates. They are poor competitors in soils because they are unable to colonize substrate occupied by other organisms (Martin and Hancock 1986). Chen et al. (1988) propose that high microbial activity and intense competition for nutrients limits

the saprophytic and pathogenic activities of P. ultimum resulting in disease suppression. Furthermore, they suggest that low microbial activity and lack of competition for nutrients may be the reason Pythium diseases are more common in soils with low oxygen or extreme soil temperatures.

The objective of this experiment was to compare the incidence of dieback at two growing mix temperatures (20, 30°C) and three mix moisture regimes ("no", "light", and "moderate" moisture stress levels). A mix temperature of 20°C is favourable for Douglas-fir root growth (Lavender and Overton 1972); 30°C is unfavourable (Parke 1983).

Methods

Two water baths located in a poly greenhouse at the CPFP Ltd. nursery were used to maintain the mix temperatures. Pregerminated seed was sown into glass test tubes containing three different growing mixes: (1) 100% steam sterilized (120°C for 40 minutes) PVM, (2) 80% (by volume) sterilized PVM and 20% dieback medium, and (3) 100% dieback medium. A known weight of air-dry medium was added to each test tube. Varying amounts of water were added to the test tubes to establish three moisture regimes corresponding to "no", "little" and "moderate" water stress. These regimes are described in Table 2. Test tubes were weighed daily and water added as needed to maintain each regime. The filled test tubes were placed in the greenhouse water baths on June 17, 1988; germinants had emerged two days later. They were misted hourly until they lost their seed coats.

The treatments were factorially arranged in a completely randomized block design with three blocks per treatment combination. The percent dieback in each block (five test tubes per block) was recorded. Analysis of variance was conducted on transformed data (square root transformation p. 234, Steel and Torrie 1980).

Results

No germinants in any of the temperature-moisture treatment combinations grown in sterilized PVM developed needle dieback symptoms. All germinants, except those in the 30°C-moderate moisture stress treatment, looked healthy; seedlings in the 30°C-moderate stress treatment appeared desiccated i.e., wilted and with red-brown needles.

Dieback symptoms did occur in the two mixes containing dieback medium. Both media contained Pythium spp. (approx. 2000 CFU/g), Fusarium solani, Trichoderma viride and Penicillium spp. There was no significant effect of media type on dieback incidence ($p = 0.80$), therefore data from these two media were combined to investigate the effects of growing mix temperature and moisture regime on dieback incidence.

Table 2. Description of "no", "light" and "moderate" moisture regimes

Moisture regime[1/]	Water added to each test tube (mL)[2/]	% moisture (oven-dry mix)
no water stress[3/]	20	570
light stress	12	340
moderate stress[4/]	4	170

1/ Based on appearance of the medium (e.g., colour, cohesiveness, amount of water that could be extracted by squeezing the medium) and MOF guidelines for moisture regimes in container nurseries.
2/ Each test tube contained 11 g of air-dry mix.
3/ Wettest suitable for Douglas-fir.
4/ Driest suitable for Douglas-fir.

Table 3 shows the results of this analysis. Together, growing mix temperature, moisture and the interaction between temperature and moisture accounted for 38% of the total variation in dieback incidence; separately they accounted for 26%, 12% and 21%, respectively.

The interaction between moisture regime and temperature is shown in Figure 2. At the growing mix temperature of 20°C, dieback incidence was much lower (6%) in medium with a "no moisture stress" regime than in medium with "light" (50%) or "moderate stress" (65%) regimes. At 30°C, moisture regime did not affect the incidence of dieback; at "no", "light" and "moderate" stress levels the incidences of dieback were 69%, 56%, and 65% respectively.

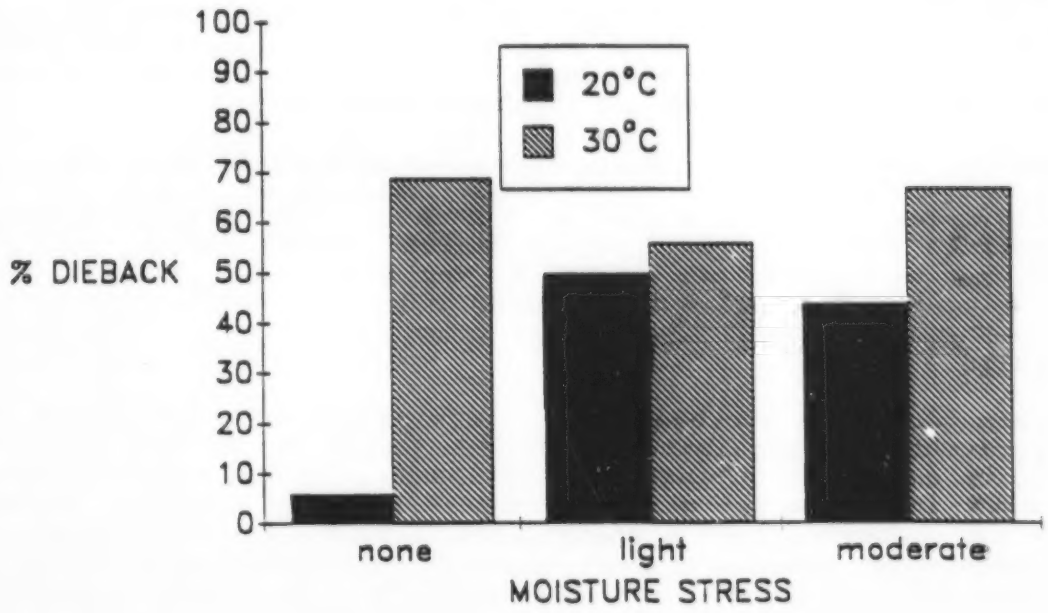
In all moisture regimes the incidence of dieback was higher at 30°C compared to 20°C. The severity of dieback symptoms was also greater at 30°C. Hoppe (1949) also found that the severity of Pythium damping-off symptoms was related to soil temperature. Corn seed inoculated with Pythium and planted into warm soil (20-24°C) favourable for the germination and growth of corn produced seedlings which were stunted but had no other damping-off symptoms. In contrast 100% of the corn seed planted into cool soil (11°C) unfavourable for corn germination was lost to the damping-off disease.

Table 3. Analysis of variance for the effects of growing mix temperature and moisture regime on dieback incidence (table values rounded to first decimal place)

Source of variation	Sum-of squares	Degrees of freedom	Mean-square	F-ratio	Prob.
Temp (T)	20.4	1	20.4	20.4	0.000
Moisture (M)	9.2	2	4.6	4.6	0.020
T*M	16.4	2	8.2	8.2	0.002
Error	31.0	30	1.0		
Total	**77.0**	**35**			

In our experiment root temperatures below the optimum for Douglas-fir root growth were not tested. It is likely that temperatures lower than optimum, particularly those below 15°C (Lavender and Overton 1972) might increase the incidence of dieback. Leach (1947) showed that Pythium-induced damping-off of horticultural crops was severest at soil temperatures unfavourable for host root growth.

Figure 2. The effects of growing mix temperature and moisture on the development of dieback.



GROWING MIX pH

Introduction

In the first year of research we observed a high incidence (20-80%) of dieback in an unrelated experiment at the UBC nursery. We obtained permission to sample the growing mix from their experiment. As reported earlier (Husted and Barnes 1987), the incidence of dieback was highest (80%) in growing mix which was not limed. Therefore, we decided to investigate the effect of growing mix acidity (pH) on dieback.

Methods

A growing mix consisting of 80% sterilized PVM and 20% dieback medium was subdivided; dolomite (Green Valley 10 mesh) was added at 0, 9 and 19 g/L to create three mixes with initial pH values of 4.0, 5.4 and 6.1, respectively. No micronutrient or N-P-K

fertilizers were added to these mixes. The experiment was a completely randomized block design with three styroblocks (313) per dolomite treatment.

The styroblocks were sown with Douglas-fir seed (SL# 7276) and placed in a poly greenhouse. The germination percentage and incidence of dieback were recorded for each styroblock six weeks after sowing. Percent data were transformed (square root transformation; p. 234, Steel and Torrie 1980) and analysis of variance was used to examine treatment effects.

Growing mix acidity (pH), electrical conductivity (EC) and available calcium were measured at the time of sowing and six weeks later. These analyses were made on water extracted from a saturated paste of the growing medium (Warnke 1986). Calcium analyses were performed by Ms. A. Van Niekerk, Forestry Canada, using atomic absorption techniques.

Results

Germination rate averaged 95%. Growing mix pH rose during the experiment by approximately 1.0 unit (Table 6); EC and available calcium decreased during the experiment. Initial growing mix pH did not influence the germination percentage ($p = 0.88$). Growing mix pH, however, had a significant effect ($p < 0.001$) on the incidence of dieback. Mean dieback incidences for initial pH values of 4.9, 5.4, and 6.1 were 93, 10, and 3 percent, respectively (Table 6). Mean values for pH 5.4 and 6.1 were not significantly different ($p = 0.15$). To confirm the effect of low pH on dieback, this treatment was repeated in six styroblocks using the same seedlot and mix (no dolomite). Dieback incidence was greater than 80% in each of these six blocks.

Similar effects of pH on diseases caused by *Pythium* have been reported in the horticultural literature (Lewis and Lumsden 1984, Kao and Ko 1986a). *Pythium* species appear not to be sensitive to soil pH within the range that supports plant growth (Cook and Baker 1983). Therefore, the influence of pH on *Pythium* disease incidence has been attributed to host growth responses (Griffin 1958) or to increases in soil bacterial populations (Elad and Chet 1987). Values of pH in the 6.0 to 7.0 range favour bacterial growth. The presence of bacteria along the roots of plants susceptible to *Pythium* has been correlated with reduced invasion of the root systems by *Pythium* (Elad and Chet 1987). These rhizosphere bacteria are thought to compete with *Pythium* for nutrients.

Oospore germination of *Pythium* is affected by exogenous nutrients (Howell and Stipanovic 1980). Nutrients supplied by root exudates stimulate oospore germination in the rhizosphere. Rhizosphere bacteria may compete with germinating oospores for carbon or nitrogen and thereby suppress germination of *Pythium*. Bacterial growth may also stimulate the lysis or degradation of *Pythium* oospores. Qian and Johnson (1988) reported that the

lysis of Pythium oospores increased in soils with pH values favourable for bacteria and actinomycete growth. They suggested that antibiotics and enzymes produced by bacteria and actinomycetes were responsible for this effect.

Table 4. Dieback incidence, initial(time of sowing) and final (six weeks after sowing) values of pH, electrical conductivity (EC) and calcium (Ca) for three levels of dolomite amendment to the growing mix. Values are the mean of three replicates.

Dolomite (g/L of mix)		pH	EC (µmhos/cm)	Ca (ppm)	Dieback (%)
0	initial	4.0	571	3	93
	final	4.9	66	2	
9	initial	5.4	630	41	10
	final	6.5	118	9	
19	initial	6.1	653	41	3
	final	6.8	132	16	

Growing mix pH and calcium availability are related.; low pH growing mixes generally have low calcium levels. Calcium is reported to increase plant vigour, root growth and resistance to diseases (Huber 1981). Calcium may also influence the germination or lysis of spores. Kao and Ko (1986a,b) reported that germination inhibition of sporangia of Pythium splendens in several different soil types was associated with high soil calcium and large total microbial populations.

According to Glen Matthews (MOF), it is not unusual for the pH values of growing mixes to be low (around 4.0) for several weeks after sowing. The results of this experiment show that low pH favours dieback. It is possible that uneven incorporation of dolomite during mixing of nursery medium may be responsible for the patchy distribution of dieback observed in container nurseries.

Further research should be conducted on growing mix pH.

(1) Our experiment showed that pH had a significant effect on dieback incidence. However, we need to know more about this

relationship between pH 4.0 and 5.4. Is the relationship linear or curvilinear? Is there a threshold level of pH?

(2) We also need to determine the calcium requirement for optimum root growth of Douglas-fir germinants in this pH range. The calcium requirement for root growth increases in acid growing medium (Marschner 1986). For example, maximum root growth of soybean plants requires 1 μm of calcium in the external solution at pH 5.6, compared with 50 μm at pH 4.8 (Lund 1970).

I. FUNGICIDE CONTROL OF DIEBACK

INTRODUCTION

Three systemic fungicides with the trade names Truban, Subdue and Aliette have been used to control root rot diseases caused by Pythium and Phytophthora, a genus closely related to Pythium (Bielenin and Jones 1988, Locke et al. 1983, Sanders et al. 1983, Stephens and Stebbins 1985). The active ingredients in these fungicides are ethazol, metalaxyl and fosetyl-Al, respectively. The objective of the following experiment was to test the efficacy of these compounds used as soil drenches in suppressing needle dieback and stunting.

METHODS

Each fungicide was applied at two rates:

(1) Aliette 80w at 6.0 and 12.0 g/10 L water,

(2) Truban 30w at 6.0 and 12.0 g/10 L of water, and

(3) Subdue 2E at 0.8 and 1.6 mL/10 L of water.

These rates were selected after reviewing results of fungicide applications to peat:vermiculite mixes (Benson 1984, Fungicide and Nematicide Tests 41:171,179, Marais 1986). The control treatment was water.

Each treatment was applied to three growing mixes: (1) 100% sterilized PVM, (2) a mixture of 10% (by volume) dieback medium and 90% sterilized PVM, and (3) a mixture of 70% dieback medium

and 30% sterilized PVM. Styroblocks (313) were loaded with growing mix, drenched with the fungicides, and allowed to drain for a day before sowing Douglas-fir seed. Fifty mL of the fungicide solution (approximately 1000mL/L of mix) was applied to each styroblock cavity.

The experiment was a completely randomized block design with a factorial arrangement of fixed treatments. Each fungicide was applied to three styroblocks. The percentage of dieback in each block was transformed (arcsin transformation p. 236, Steel and Torrie 1980) before analysis of variance.

RESULTS

Fungicide application, growing mix, rate of fungicide application, and the interaction between fungicide and mix type had statistically significant effects on the incidence of dieback in this experiment (Table 5).

Table 5. Analysis of variance of the dieback incidence for the three fungicide treatments applied at two rates to the two growing mixes containing dieback medium.

Source of variation	Sum-of squares	Degrees of freedom	Mean-square	F-ratio	Prob.
Fungicide(F)	10745	2	5372	59	0.000
Mix(M)	560	1	560	6	0.020
Rate(R)	1392	1	1392	15	0.001
F*M	1131	2	566	6	0.007
F*R	530	2	264	3	0.073
R*M	28	1	28	1	0.582
F*M*R	327	2	163	2	0.186
Error	2176	24	91		

Mean treatment values (transformed back to the original scale of measurement) are shown in Table 6. Aliette was the only fungicide to reduce the incidence of dieback relative to the control. In the 10:90 mixture of dieback medium and sterilized PVM both rates of Aliette were equally effective. In the 30:70 mixture, only the higher application rate (12.0 g/10 L water) reduced dieback incidence compared to the control.

Table 6. Mean incidence of dieback (%) for the control and three fungicide treatments (means are in the original measurement scale).

Fungicide	Growing Mix		
	Sterilized PVM	Dieback and Sterilized PVM (10:90)	Dieback and Sterilized PVM (70:30)
control	0	42.6	11.0
Aliette			
6 g/10 L	0	3.2	17.1
12 g/10 L	0	3.2	4.4
Truban			
6 g/10 L	6.4	96.8	69.8
12 g/10 L	9.0	55.1	44.5
Subdue			
0.8 mL/10 L	1.6	62.8	30.2
1.6 mL/10 L	4.4	43.9	21.1

Aliette is not registered in Canada for use in nurseries. However, lack of registration may not be a problem for its use. Phosphorous acid, a hydrolysis product of fosetyl-Al, has been shown to be as effective as fosetyl-Al in the control of phytophthora root rots (Fenn and Coffey 1984, Rohrback and Schenck 1985). Aliette probably has a direct fungitoxic effect on Phytophthora spp. (Farih et al. 1981, Fenn and Coffey 1984). Farih et al. (1981) demonstrated that the degree of inhibition of Phytophthora by fosetyl-Al in pH-adjusted solutions (pH 6.0) was related to the concentration of fosetyl-Al. However, they did not rule out the possibility of a minor indirect pH effect.

Truban and Subdue did not reduce the incidence of dieback compared to the control in either mix or at either rate of application. However, the incidence of dieback was lower in the 70:30 mix of dieback medium and sterilized PVM than in the 10:90 mix. The appearance of needle dieback symptoms in the sterilized PVM treated with Truban and Subdue are difficult to explain. It

is possible that symptoms of needle dieback and phytotoxicity are similar.

This experiment shows that fosetyl-Al but not ethazol or metalaxyl used as a soil drench reduces the incidence of dieback. Further research should be conducted to determine:

(1) the efficacy of fosetyl-Al after seedlings have germinated, particularly after dieback symptoms (greening of stems, needle tip dieback) are observed in the greenhouse crop, and

(2) if phosphorous acid is as effective as fosetyl-Al for the control of Pythium root rots.

J. CONCLUSIONS

Dieback of container-grown Douglas-fir germinants is caused by Pythium, a minor root pathogen. There are numerous reports of subclinical damage caused by Pythium spp. (Salt 1979, Kobriger and Hagedorn 1984, Hodges 1985, Hershman et al. 1986, Stanghellini and Kronland 1986). Subclinical damage is characterized by stunting and growth losses in plants which have normal-appearing roots with no external symptoms of root rot. Minor root pathogens are generally restricted to juvenile root tissues such as root hairs, root tips or cortical cells (Salt 1979). Pythium seriously reduces root hair development of Douglas-fir germinants. Root hairs are a significant proportion (50%) of the root surface area of young seedlings (Kozlowski and Scholtes 1948). Therefore, root hair damage may seriously reduce water and nutrient absorption by Douglas-fir germinants.

The degree of damage caused by minor root pathogens typically depends on the growing environment and host plant vigour (Salt 1979). Seedling age, growing mix temperature, moisture and acidity (pH) are important factors influencing the incidence of dieback in container nurseries. Seedlings are most susceptible to dieback in the first weeks after germination. Eighty to ninety per cent of one-week-old germinants transplanted into growing medium containing the dieback pathogen develop dieback symptoms; in contrast, eight-week-old seedlings transplanted into dieback medium do not develop dieback symptoms. Other conifer species do not appear to be susceptible to the dieback pathogen even during the germination period.

The strong influence of growing mix temperature, moisture and pH (section G) on the incidence of dieback shows that cultural management of the growing mix environment will reduce the

incidence of dieback even if Pythium is present. Creating environmental conditions favourable for Douglas-fir root growth and for microbes which will compete with Pythium for root exudates minimizes dieback damage. Methyl bromide fumigation of the growing mix and styroblocks will also reduce dieback damage. However, health, safety, and ecological (i.e., removing all soil organisms both "good" and "bad") concerns make the fumigation option less desirable than cultural or biological treatments for the control of diseases caused by minor root pathogens.

Fosetyl-Al or its hydrolysis product phosphorous acid may be useful for the control of dieback. However, further research should be conducted (1) to determine the efficacy of fosetyl-Al after seedlings have germinated, particularly after dieback symptoms (greening of stems, needle tip dieback) appear in a greenhouse crop, and (2) to compare the efficacy of phosphorous acid to fosetyl-Al for the control of Pythium root diseases.

We believe that more research should be conducted to determine the effects of growing mix pH and calcium on Douglas-fir root growth and dieback incidence. It is not unusual for the pH values of growing mixes to be low (approx. 4.0) for several weeks after sowing. Our results show that low pH favours dieback. Possibly uneven incorporation of dolomite during the preparation of nursery peat:vermiculite medium is responsible for the patchy distribution of dieback observed in container nurseries.

Although we showed that pH has a significant impact on dieback incidence, we need to know more about this relationship between pH values of 4.0 and 5.4. Is the relationship linear or curvilinear? Is there a threshold level of pH below which dieback incidence increases? We also need to determine the calcium requirement for optimum root growth of Douglas-fir germinants in this pH range. The calcium requirement for root growth increases in acidic growing medium (Marschner 1986). For example, maximum root growth of soybean plants requires 1 μm of calcium in the external solution at pH 5.6, compared with 50 μm at pH 4.8 (Lund 1970).

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APPENDIX A

METHODS FOR KOCH'S POSTULATE TESTING

Seed Preparation

Douglas-fir seeds from seedlot number (SL#) 7276 were sterilized with 15% hydrogen peroxide for 12 hours; rinsed thoroughly with sterilized water; and soaked another 12 hours in sterilized water. The germination rate of sterilized seed was similar to that for nonsterilized seed. *Pythium* was not isolated from sterilized or nonsterilized seed.

Growing Mix and Containers

Douglas-fir seeds were sown into new 313 styroblock cavities (51 cc volume) filled with sterile peat:vermiculite media (PVM) containing lime and osmocote. PVM was sterilized by autoclaving (40 minutes at 122°C). PVM moisture content prior to autoclaving was approximately 200%.

Isolation of Pythium

Four *Pythium* organisms were isolated from oospores obtained from cleared roots of dieback seedlings collected in various 1986/87 dieback research trials. These isolations were made on medium selective for *Pythium*, water agar and V8-juice agar (VJA). The isolates were numbered 729, 7111, 7116 and 7126, respectively by M.B. Research and Development, Sidney, B.C. In vitro testing of these isolates indicated they were pathogenic. Douglas-fir germinants were introduced into *Pythium* cultures for 24 to 48 hours; then the cleared roots were examined for tissue invasion by *Pythium*. All isolates invaded the root tissue of the germinants in the laboratory medium.

Inoculum Production

Four inoculation methods were tried. Two successful methods are described below. In both cases, the inoculum consists of hyphae, oospores and resting spores.

Method 1. Inoculum slurry mixed with growing medium

Pythium isolates were cultured on VJA by M.B. Research Development. Six petri plates of each isolate were mixed with

500 mL of 0.01% potato dextrose broth (PDB), squeezed through cheesecloth to homogenize the inoculum mixture, and added to six litres of sterilized peat:vermiculite mix (PVM). A control was prepared by mixing 500 mL of PDB with six litres of sterilized PVM. The inoculated PVM was incubated for 48 hours in a sealed plastic bag at 21°C, handloaded into styroblocks, covered and incubated for a further week, and then sown with two seeds per cavity. Seventy styroblock cavities were inoculated for each treatment and control.

The inoculum potential of the treatments and control were estimated at sowing. The control had no Pythium. The inoculation treatments had 7,000 to 10,000 colony-forming units (CFU) of Pythium per gram of medium (fresh weight).

Method 2. Inoculum slurry injection

Sterile PVM was handloaded into 313 styroblock cavities. Ten VJA plates of each inoculum were homogenized in 500 mL of sterile water in a Waring blender. Twenty mL of VJA were added to each inoculum mixture. A control inoculum was prepared from V8 juice and sterile water. Twenty mL of inoculum were injected by syringe into each styroblock cavity. Twenty cavities were inoculated for each treatment and control. Inoculated cavities were incubated at 21°C for 48 hours before sowing at two seeds per cavity.

Growing Regime

The seeds were germinated at 22°C day, 18°C night temperatures and 65-70% relative humidity (RH) for two weeks in a growth chamber with a 16h-photoperiod. Photosynthetically active radiation (PAR) ranged from 200 to 500 microeinsteins per square metre second during the day. A hydrothermograph was placed inside the growth chamber a week prior to the trial to ensure the chamber was maintaining programmed levels of temperature and humidity.

At the end of the germination period, the seedlings were stressed for 48 hours by increasing the maximum day temperature to 28°C and decreasing the relative humidity to 40%. The night temperature and relative humidity were 12°C and 90% respectively. These stress conditions were similar to nursery temperatures and humidities during the development of dieback in 1986. Following the stress period, seedlings were returned to the initial growing regime.

Monitoring

Samples of seedlings were collected from each inoculum treatment 28 days from sowing. *Pythium* invasion of root tissue was determined by direct microscopic examination of cleared roots and by plating surface sterilized and cleared roots on medium selective for *Pythium*. Cleared roots were prepared from roots fixed with lactophenol and stained with cotton blue (Linden 1929). Tissue was surface sterilized with sodium hypochlorite and alcohol.

APPENDIX B

EXPERIMENTS CONDUCTED FOR ALTERNATIVE EXPLANATIONS OF STERILIZATION EFFECT

WATER-SOLUBLE TOXINS

Methods

Sixty 313 styroblock cavities were filled with sterilized PVM and sown at two seeds per cavity with Douglas-fir SL# 7276. Half of these cavities were watered with sterile water (distilled water passed through a 0.22 micron filter); the other half with a water-soluble filtrate from dieback medium. The filtrate was prepared by adding 1500 mL of dieback medium to 750 mL of distilled water. This slurry was allowed to stand for two hours and then filtered through a series of filters down to 0.22 microns to remove all organisms. The filtrate was not stored; fresh filtrate was prepared for each watering. The seedlings were germinated at 25°C day/21°C night, 65% relative humidity and a 16 hour photoperiod.

Results

Germination rates were low: 23% and 53% respectively for seedlings moistened with sterile water and dieback filtrate. The low germination rate was attributed to a period of high temperatures during germination. We tried to protect the filtrate trial by covering the seed with moist towels but still had reduced germination. The high temperatures were unavoidable as another dieback trial (Koch's postulates) which required a stress treatment (28°C) was in progress in the same growth chamber.

None of the seedlings that germinated in either the sterile water or filtrate treatments showed dieback symptoms. This trial was repeated with a more favourable temperature (20°C). Again no dieback symptoms appeared in seedlings watered with filtrate or sterile water, confirming the results of an earlier experiment (page 54, Husted and Barnes 1987), i.e., a water-soluble toxin is not a major cause of dieback.

ABIOTIC FACTORS

Introduction

The suppression of dieback in sterilized medium strongly suggests a biological agent causes dieback. However, many chemical and physical changes accompany the sterilization process (Mulder 1979). It is possible that these changes are responsible for the reduced incidence of dieback after medium sterilization. Two of the 1986 hypotheses proposed to explain dieback involved ammonium toxicity and excessive nitrogen fertilization. Sterilization is known to alter nutrient concentrations in growing mixes (Mulder 1979); therefore, proponents of the nitrogen hypotheses suggested that sterilization might decrease the incidence of dieback by lowering ammonium or available nitrogen concentrations in the growing mix.

Methods

Two experiments were conducted to confirm that the sterilization effect is not due to physical or chemical changes in the medium. In the first experiment, Douglas-fir seed was sown into two different growing media: (1) a mix consisting of 10% (by volume) dieback medium and 90% sterile PVM and (2) a control mix of 100% sterile PVM. The styroblocks were placed in a Forestry Canada greenhouse in November 1987. High pressure sodium lights were used to supplement the lighting. The growing regime was a 16 hour photoperiod with 21°C day/15°C night temperatures in November 1987. The relative humidity fluctuated with an average value of 65% to 70%. Two weeks after germination the seedlings were stressed by raising the blocks close to the high pressure sodium lights so that the air temperature was increased to 28°C. No fertilizer was applied for the duration of the trial.

In the second experiment the effect of sterilization on ammonium and nitrate concentrations was investigated. High ammonium concentrations or ammonium to nitrate ratios have been reported in the medium of dieback seedlings (Husted and Barnes 1987). KCL-extractable ammonium and nitrate concentrations were determined before, immediately after, and two, five, and seven weeks after steam sterilization (122°C for 40 minutes) of dieback medium. After sterilization the medium was vented at room temperature (approx. 20°C). Three samples of medium were combined (at each sample date), thoroughly mixed and stored at 1°C prior to analysis. Analyses were conducted by MacMillan Bloedel, Nanaimo, B.C.

Results

Twelve per cent of the seeds sown in sterile PVM diluted with 10% dieback medium developed dieback symptoms. No dieback symptoms appeared in the 100% sterile PVM. The induction of dieback with a small volume of dieback medium (10% v/v) strongly suggests that dieback suppression in sterilized soils is not due to any changes in abiotic factors accompanying medium sterilization.

Nitrate and ammonium analyses before and after sterilization are shown in Table 1. For several weeks after sterilization, ammonium levels were higher than pre-sterilization levels. This trend has been observed by other researchers (Rovira 1976; Mulder 1979) and in our earlier research (Husted and Barnes 1987). From two to five weeks after sterilization (the period during which dieback symptoms appear in nonsterile medium), ammonium concentrations decreased to pre-sterilization levels. Total available nitrogen (nitrate plus ammonium) was not changed significantly by sterilization. Sterilization does not appear to reduce the incidence of dieback by lowering ammonium or available nitrogen levels during seed germination.

Table 1. KCL-extractable ammonium and nitrate (ppm dry weight basis) before and after autoclaving

Time of sample		ammonium	nitrate
before autoclaving		107	949
# of weeks after autoclaving	0	188	893
	2	175	906
	5	96	1002
	7	10	922

EFFECT OF STERILIZATION ON MICROBIAL POPULATIONS

Introduction

Plant growth sometimes improves after medium sterilization due to the stimulative action of some non-pathogenic organisms (Altman 1970, Altman and Tsue 1965) or the antagonism of certain soil organisms, particularly fluorescent pseudomonads and Trichoderma spp. to root pathogens (Weindling 1932; Papavizas 1986; Stutz, Defago and Kern 1986). Elad and Chet (1987) reported that several pseudomonad species appeared to inhibit the germination of Pythium aphanidermatum oospores by competing for nutrients. Howell and Stipanovic (1980) found Pythium-induced damping-off in cotton was inhibited by an antibiotic produced by Pseudomonas fluorescens. Sterilization can increase the populations of these antagonistic organisms (Ridge 1986; Ridge and Theodorou 1972).

Methods

Dieback medium (pest:vermiculite plus some perlite and dolomite) was autoclaved for 40 minutes at 122°C. Moisture content before sterilization was 140%. Microbial analyses were conducted: (1) prior to autoclaving, (2) immediately after autoclaving and (3) after two weeks of venting (the usual planting time for our trials). The medium was vented at 21°C.

Microbial Analyses

Microbial analyses of seedling roots and the growing medium were conducted by M.B. Research and Development Ltd., Sidney, B.C. Organisms were cultured from cleared root tissue, surface sterilized roots and the growing medium. Cleared roots were prepared from roots fixed with lactophenol and stained with cotton blue (Linden 1929). Tissue was surface sterilized with sodium hypochlorite and alcohol. Tissues were plated at different dilutions on malt extract-peptone medium, PDA (potato dextrose agar), blood agar, and VJA (V8-juice agar) with antibiotics for a comparison of microbial populations. Pythium was isolated on modified PDA medium. The growing medium was plated on malt-extract-peptone medium, PDA and VJA.

Fungal genera were identified by colony morphology and reproductive structures. Bacteria were isolated from the colony and suspended in heart infusion broth, then identified to genus level by morphology and biochemical tests. Results are reported in colony forming units (CFU) per gram (fresh weight) of tissue or medium.

Results

The microbial counts before and after sterilization are shown in Table 2. No Pythium was detected immediately after sterilization. However, two weeks later, the count of Pythium spp. had increased to a level 100 times greater than the pre-sterilization level. The rapid growth of these Pythium spp. suggests that they were saprophytic and not pathogenic. Similarly high counts of Pythium were also found in the sterilized medium used in the first Koch's postulate experiment. However, no dieback occurred in this test indicating the Pythium were saprophytic.

Moisture content of the medium before sterilization influences the effectiveness of the sterilization treatment. At a moisture content of 200% instead of the 140% used in this trial there is no Pythium two weeks after sterilization.

Table 2. Microbial counts (CFU/g fresh weight of medium) before, immediately after, and two weeks after autoclaving

Time	Organisms	cfu/g
Before sterilization	Pythium	200
	Penicillium	1 000
	Streptomyces	2 000
Immediately after sterilization	Penicillium	6 200
Two weeks after sterilization	Pythium	20 000
	Penicillium	120 000
	Trichoderma	60 000

It is very difficult to identify Pythium to the species level. Until the Pythium can be categorized as saprophytic or pathogenic, counts of Pythium spp. cannot be used to predict the

potential for dieback. To complicate an already complex situation, it is known that some saprophytic Pythium spp. are mycroparasites of pathogenic Pythium spp. (Martin and Hancock 1987).

The increases in Penicillium and Trichoderma populations are consistent with previous analyses conducted in 1986/87 research. Sterilization of the medium results in "blooms" of primary colonizers (Penicillium, Trichoderma, and Pseudomonas) which may inhibit root pathogens. Pathogenic Pythium are in general weak competitors compared to saprophytic Pythium spp. or primary colonizers (Krupa and Dommergues 1979).

These results suggest that antagonism plays a role in the sterilization effect. In a follow-up experiment we sowed Douglas-fir seeds in either sterilized (120°C for 40 min.) or nonsterile PVM mixed with dieback medium (10% v/v). Dieback incidence for the sterilized and nonsterilized PVM were 12% and 71% respectively, indicating that the sterilized medium was more inhibitory to the dieback pathogen. Fast growing antibiotic producing fungi, such as Trichoderma spp. and Pencillium frequentans, may in some cases, only inhibit Pythium in sterile soil (Liu and Vaughan 1965). In nonsterile soil, these same organisms may be lose their ability to inhibit Pythium.